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FORMATION AND ITS CONSEQUENCES FOR
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MICROBIAL BIOFILM FORMATION AND ITS
CONSEQUENCES FOR THE CELSS PROGRAM

from

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INTRODUCTION

A major goal of the CELSS program is to provide reliable and efficient life support systems for long-duration space flights. A principal focus of the program is on the growth of higher plants in growth chambers. These crops should be grown without the risk of damage from microbial contamination. While it is unlikely that plant pathogens will pose a risk, there are serious hazards associated with microorganisms carried in the nutrient delivery systems and in the atmosphere of the growth chamber.

Our experience in surface microbiology has shown that colonization of surfaces with microorganisms is extremely rapid even when the inoculum is small. After initial colonization extensive biofilms accumulate on moist surfaces. These microbial films metabolize actively and slough off continuously to the air and water. During plant growth in the CELSS program microbial biofilms have the potential to foul sensors and to plug nutrient delivery systems. In addition both metabolic products of microbial growth and degradation products of materials being considered for use as nutrient reservoirs and for delivery are likely sources of chemicals known to adversely affect plant growth.

RESEARCH ACCOMPLISHED

In the first phase of our research on this project, emphasis was placed on the mechanisms of the adhesion of bacteria to a variety of surfaces. We found that some surfaces are more susceptible to the formation of the biofilms than others. In our study we used the bacterium Pseudomonas aeruginosa, which is common in water, and is an opportunistic pathogen of plants. We found that this bacterium survives for long periods in biofilms. In addition, when it is protected by its own exopolymers on surfaces it seems to be totally resistant to disinfection by iodine. Some of these results are discussed in more detail below.

In order to examine the adhesion of bacteria to different surfaces, stainless steel and titanium were sputter coated onto glass cover slips using an ion beam sputtering apparatus designed and built in Harvard's Materials Research Laboratory. In addition we have built a computer enhanced image analysis system. It has proved to be an important tool in our investigation of bacterial adhesion. We have employed this technique to investigate the adhesion of bacteria to surfaces in flowing systems. In comparisons of adhesion to glass, titanium and stainless steel, bacteria were found to attach to all surfaces rapidly. Within 100 hours all three surfaces were totally coated with bacteria.

In a separate series of experiments, the attachment of P. aeruginosa to welded and non-welded sites was examined. In past case histories, weld sites have been associated with active microbial growth. The reasons for this preferential bacterial activity at weld sites are not known. We inoculated stainless steel coupons containing welds in the center with bacteria isolated from water. In all cases our analysis showed much greater bacterial activity on the weld surfaces. These data suggest a potential hazard for the CELSS program, since welding material is rich in chromium. The welds rapidly leach metals during bacterial

growth. In the next phase of this research we intend to expand this work to determine the nature of the leaching metals and the rate of dissolution. We also plan to study the rates of biofilm formation and metal leaching using a number of different metals.

We found that some bacteria survive surprisingly well when attached to surfaces, while others die off rapidly. For example, Staphylococcus aureus was not able to survive for more than a week on stainless steel, while Pseudomonas survived and grew for at least five months. The activity was maintained at a wide temperature range from 10° to 37°C.

We attempted to determine the mechanism of survival of bacteria on surfaces. Our experiments showed that a hydrophobic interaction is involved. The formation of the biofilms appears to be controlled by synthesis of a binding polymer in response to the recognition of the substratum by the bacterium. We are currently attempting to determine if different physical or chemical conditions on the substratum alter the chemistry and binding characteristics of the bacterial exopolymer.

We compared the susceptibility of free and attached bacteria to disinfection by iodine. In all cases we used water flowing past stainless steel sputter-coated on glass slides. Adhesion was measured using computer enhanced image analysis. The bacteria free in the water were rapidly killed by low concentrations of iodine. However, once a biofilm formed it could survive iodine levels as high as 100 ppm. Furthermore, the biofilm continued to grow in the presence of iodine. In the next phase of this research we intend to determine if there are compatible disinfectants or surfactants that might prevent biofilm formation. We also intend to investigate materials treated to prevent biofilm formation.

The research summarized above has been presented at scientific meetings or published in scientific journals. A number of papers are in preparation. The publications are listed below.

PUBLICATIONS SUPPORTED BY THIS PROJECT

1. Boyle M, Ford T, Mitchell R, Maki JS (1990). Survival of pathogenic bacteria under nutrient starvation conditions. Proc. 20th Intersociety Conf. Environmental Systems. SAE Technical Paper 901381.
2. Boyle J, Ford T, Maki JS, Mitchell R (1991). Biofilms and the survival of opportunistic pathogens in recycled water. Waste Management & Research 9:465-470.
3. Joyce E, Hogan D, Ford T, Mitchell R (1992). Substratum effects on biofilm formation. Proc. American Society for Microbiology, 92nd Annual Meeting, New Orleans.
4. Maki JS, Joyce EA, Mitchell R (1992). Inhibition of protein synthesis and adhesion

of a copiotrophic bacterium during nutrient deprivation. Proc. American Society for Microbiology, 92nd Annual Meeting, New Orleans. Abstract Q125.

5. Ford T, Wagner A, Mitchell R (1993). Sputter-coating technology for preparation of thin, optically transparent films of stainless steel and titanium. (In preparation.)
6. Ford T, Joyce E, Mitchell R (1993). Use of image analysis to study attachment characteristics of bacteria to stainless steel, titanium and glass. (In preparation.)
7. Ford T, Mitchell R (1993). Iodine resistance of attached films of bacteria. (In preparation.)
8. Maki JS, Joyce EA, Mitchell R (1993). Adhesion of Pseudomonas aeruginosa to surfaces under static and flowing conditions. (In preparation.)
9. Maki JS, Mitchell R (1993). Use of transposition mutation to investigate on adhesion of copiotrophic bacteria. (In preparation.)

FUTURE RESEARCH

In the first phase of this research we demonstrated the ability of bacteria to form biofilms on a wide range of surfaces. The bacteria survived for months on inert substrata, protected by their exopolymers. We developed computer enhanced image analysis techniques to investigate adhesion and survival of bacteria on surfaces in contact with flowing water. We used this method to demonstrate that biofilms are highly resistant to disinfection with iodine.

In the next phase of our research we plan to expand our investigation to include the following objectives:

1. To evaluate formation of biofilms on the surfaces of materials being considered for use in CELSS plant growth chambers and nutrient delivery systems;
2. To assess the production of metabolites by microorganisms in biofilms that are potentially toxic to plants;
3. To evaluate the release of toxic chemicals due to the interaction of biofilms with materials under consideration for use in the CELSS program;
4. To determine the effect of changes in environmental parameters on biofouling of the nutrient delivery system;
5. To determine how disinfectants or surfactants in the water or modification of surfaces will prevent biofilm formation.